

Dissecting the roles of β -arrestin2 and GSK-3 signaling in 5-HT1BR-mediated perseverative behavior and prepulse inhibition deficits in mice

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Supplemental Results

Results

No effect of SB216763 on low-dose RU24969-induced changes in behavior

In Experiment 1, 3 mg/kg RU24969 treatment increased distance traveled across pretreatment groups ($F_{(1,72)} = 12.12$; $p < .001$; S1A Fig). SB216763 pretreatment had no effect on distance traveled. RU24969 also reduced time spent resting across pretreatment groups for the low dose ($F_{(1,72)} = 14.55$; $p < .0005$; S1B Fig), whereas SB216763 had no effect on rest time. 3 mg/kg RU24969 had no effect on spatial d (S1C Fig).

No effect of AR-A014418 on high-dose RU24969-induced changes in behavior

In the open field, RU24969 treatment increased total distance traveled across pretreatment groups ($F_{(1,78)} = 85.57$; $p < .0001$; S2A Fig). AR-A014418 pretreatment did not interact with RU24969 treatment or have a main effect on distance traveled. RU24969 also reduced time spent resting across pretreatment groups ($F_{(1,78)} = 136.45$; $p < .0001$; S2B Fig), while AR-A014418 had no effect on rest time. RU24969 reduced spatial d across pretreatment doses ($F_{(1,75)} = 5.54$; $p < .05$; S2C Fig). AR-A014418 also reduced spatial d ($F_{(2,75)} = 3.91$; $p < .05$; S2C Fig) at both the 10 mg/kg and 20 mg/kg doses, but did not interact with RU24969 treatment to affect spatial d . Virtually no vertical activity was observed in Experiment 4 (data not shown); thus, statistical analysis was not performed. RU24969 treatment increased startle amplitude overall ($F_{(1,78)} = 20.38$; $p < .0001$; S2D Fig), whereas AR-A014418 had no effect on startle.

RU24969 treatment decreased PPI overall ($F_{(1,78)} = 47.24$; $p < .0001$; S2E Fig), whereas AR-A014418 had no effect on PPI.

Relationship between saline and RU24969-treated activity levels in the open field within *Arrb2* genotype

None of the genotypes had a significant correlation between distance traveled in the saline and 3 mg/kg RU24969 conditions (WT: $r = .27$; 95% CI: $-.11$ to $.58$; $p = .16$; HT: $r = .16$; 95% CI: $-.23$ to $.50$; $p = .42$; KO: $r = .30$; 95% CI: $-.09$ to $.61$; $p = .12$) or between the saline and 10 mg/kg RU24969 conditions (WT: $r = .017$; 95% CI: $-.35$ to $.38$; $p = .93$; HT: $r = .27$; 95% CI: $-.11$ to $.58$; $p = .16$; KO: $r = .027$; 95% CI: $-.35$ to $.40$; $p = .89$). This lack of relationship was further supported by simple linear regression models assessing RU24969-induced distance traveled based on saline-induced distance traveled. For *Arrb2* WT mice, there was no significant regression between saline and 3 mg/kg RU24969 distance traveled ($F_{(1,27)} = 2.08$; $p = .16$; $r^2 = .07$) or between saline and 10 mg/kg RU24969 ($F_{(1,27)} = .01$; $p = .93$; $r^2 = .0003$). Likewise for HT mice, there was no significant regression between saline and 3 mg/kg RU24969 ($F_{(1,26)} = .69$; $p = .41$; $r^2 = .03$) or 10 mg/kg RU24969 ($F_{(1,27)} = 2.15$; $p = .15$; $r^2 = .07$). Similarly for KO mice, there was no significant regression for 3 mg/kg ($F_{(1,25)} = 2.55$; $p = .12$; $r^2 = .09$) or 10 mg/kg RU24969 ($F_{(1,26)} = .02$; $p = .89$; $r^2 = .001$). Furthermore, the effect size of 10 mg/kg RU24969 treatment on distance traveled was substantially larger in WT mice ($d = -3.43$) than in HT ($d = -2.54$) or KO ($d = -2.53$).

None of the genotypes had a significant correlation between time spent resting in the saline and 3 mg/kg RU24969 conditions (WT: $r = .083$; 95% CI: $-.29$ to $.44$; $p = .67$; HT: $r = .011$; 95% CI: $-.36$ to $.38$; $p = .95$; KO: $r = .058$; 95% CI: $-.33$ to $.43$; $p = .78$) or between the

saline and 10 mg/kg RU24969 conditions (WT: $r = .023$; 95% CI: $-.340$ to $.380$; $p = .91$; HT: $r = .291$; 95% CI: $-.078$ to $.589$; $p = .12$; KO: $r = .027$; 95% CI: $-.350$ to $.396$; $p = .89$). This lack of relationship was further supported by simple linear regression models between saline and RU24969-treated conditions. For WT mice, there was no significant regression between saline and 3 mg/kg RU24969 time spent resting ($F_{(1,27)} = .19$; $p = .67$; $r^2 = .007$) or between saline and 10 mg/kg RU24969 ($F_{(1,27)} = .07$; $p = .79$; $r^2 = .003$). Similarly for HT mice, neither 3 mg/kg ($F_{(1,26)} = .003$; $p = .95$; $r^2 = .0001$) nor 10 mg/kg ($F_{(1,27)} = .05$; $p = .83$; $r^2 = .002$) had significant regressions. Finally, KO mice did not have a significant regression for 3 mg/kg ($F_{(1,25)} = .09$; $p = .77$; $r^2 = .003$) or for 10 mg/kg RU24969 ($F_{(1,26)} = .49$; $p = .49$; $r^2 = .02$). Furthermore, the effect size of 10 mg/kg RU24969 treatment on rest time was larger in the WT mice ($d = 3.31$) than in the HT ($d = 2.59$) or KO mice ($d = 2.45$).